

REMARKS

Entry of the foregoing, reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.116, are respectfully requested in light of the remarks which follow.

I. Amendments to the Claims

By the foregoing claim amendments, claims 1-4 have been amended to recite that the step of isolating the inner cell mass cells by mechanical dissection into pieces does not comprise the use of immunosurgery. This amendment is supported at least at page 5, lines 7-21 of the present specification.

In addition, claim 11 has been amended to depend from claim 1 rather than from claim 7.

The amendments to the claims have been made without prejudice or disclaimer to any subject matter recited or canceled herein. Applicants reserve the right to file one or more continuation and/or divisional applications directed to any canceled subject matter. No new matter has been added, and entry of the foregoing amendments to the above-identified application are respectfully requested.

II. Response to Claim Rejections Under 35 U.S.C. § 103

A. At pages 3-8 of the Office Action, claims 1-3, 5, 6, 12, 13, 16-20, 62, 64, and 65 stand rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Thomson (2001) when taken with Thomson (1998) as evidenced by Stem Information (National Institutes of Health) when taken with Rijinders et al. and in further view of Lanzendorf et al. when taken with U.S. Patent No. 6,875,607.

This rejection is respectfully traversed, for at least the following reasons.

To expedite prosecution in the present application, and not to acquiesce to the Examiner's rejection, the claims have been amended as described above. In particular, the claims have been amended to recite that the step of isolating the inner cell mass cells by mechanical dissection into pieces does not comprise the use of immunosurgery.

Applicants respectfully submit that a person of ordinary skill in the art, at the time of filing, would not have reasonably predicted that the inner cell mass (ICM) cells could be successfully isolated by mechanical dissection into pieces without the use of immunosurgery.

Reviewing the prior art *before* the time of filing, it is apparent that the method applied for isolation of the ICM included an immunosurgical step for the removal of the trophectoderm. As apparent from Thomson et al. (1998), p. 1147 (6); Reubinooff et al. (2000), p. 403; Hovatta et al. (2003), p. 1405; U.S. Patent No. 6,875,607, col. 8, line 31 to col. 9 line 27; and Cowan et al (2004), Supplementary appendix 1, all protocols published before the time of filing included immunosurgery to facilitate isolation of the ICM by enzymatic removal of the trophectoderm. Thus considering the specificity and uniformity of the prior art, the art related to establishing hBS cell lines at the time of filing clearly and specifically taught the use of immunosurgery and did not teach or suggest how to isolate the ICM without the use of immunosurgery.

To address the Examiner's arguments as set forth at page 4, first paragraph, of the June 24, 2009 Office Action, Applicants respectfully submit that all cited prior art documents recite the use of immunosurgery to remove the trophectoderm before manual dissection of the ICM. As exemplified below, Thomson (1998), p 1147, 2nd column, #6 recites: "*The inner cell mass was removed by immunosurgery (26)*". Stem Information (NIH) and Rijnders (1998) do not specify isolation of the ICM. Thomson (2001)/US 6,200,806, column 8, line 33-43 recite the use of immunosurgery: "*For immunosurgery, blastocysts are exposed to a 1:50 dilution of rabbit anti marmoset spleen cell antiserum...*" and "*...lysed trophectoderm cells are removed from the intact inner cell mass by gentle pipetting...*" Further, Lanzendorf (2001), p 134, left column, section headed *Immunosurgery* recites: "*The trophectoderm was removed by from expanded blastocysts by immunosurgery as previously described in the mouse (15), rhesus monkey (8), and human (13)*". Finally U.S. Patent No. 6,875,607 ('607) includes immunosurgery as it recites the method at column 8, line 67, to column 9, line 22. The '607 patent does not teach or suggest that the isolation may be done with pure mechanical dissociation.

At page 4 of the Office Action, the Examiner further argues that it is obvious for the skilled artisan to substitute any method for isolation of ICM cells with another, to achieve the result of isolation of ICM cells. The Examiner states that *passaging* by mechanical dissociation is described in e.g. '607 and it therefore is obvious for the skilled artisan to apply this method to *isolate* the ICM.

In this respect, Applicants respectfully submit that in addition to the fact that none of the skilled artisans at the time of filing arrived at the claimed invention, the heterogenous

structure of the blastocyst compared to the homogenous nature of the isolated and cultured ICM makes it non-obvious to apply a method for passaging to isolating the ICM. The isolated and propagating ICM forms a relative uniform cell colony with no externally surrounding membrane or cell-structure when cultured as described in present application. The blastocyst is a heterogenous structure comprising the ICM surrounded by the zona pellucida and the trophoctoderm. As described above, all relevant prior art documents describe the use of immunosurgery to remove the trophoctoderm. The enzymatic step was included for two reasons: to physically dismantle the blastocyst structure by dissolving the basket in which the ICM is embedded and secondly to minimize the presence of trophoblasts in the subsequent culturing steps.

The present application relates to the discovery of a method by which immunosurgery is omitted and the isolation of ICM is performed by a solely mechanical dissection of the blastocyst. The technical effect of omitting the use of enzymes and antibodies allows a more economic, xeno-free and less time-consuming method.

Reviewing the literature *after* the time of filing, it seems apparent that the method as claimed in present application has become the preferred method for ICM isolation with documented advantages over the originally published immunosurgically based methods. In a comparative study, Sjogren et al. (2004) found that, despite that fact that the blastocysts used as starting material for immunosurgical isolation were morphologically superior than the group used for mechanical isolation (p. 327, 6th paragraph), the yield from the direct (mechanical) culture according to Heins et al. (2004) were more than twice as high as the yield from immunosurgically isolated cultures (p. 328, table 2). Years later, Ström et al. (2007) made corresponding observations in a comparative study with mechanical and immunosurgical isolation of the ICM, where the two groups yielded 26% and 16% viable cell lines respectively (p. 3051, abstract). Also for the purpose of a xeno-free establishment of human embryonic stem cell lines, Ellerström et al. (2006) prefers an immunosurgically free establishment.

Hence, the method recited in the present claims provides an easier method with improved user compliance and significantly improved yields.

It is therefore respectfully submitted that, at the time of filing, the available literature uniformly taught the use of immunosurgery. The present inventors experimented against this to establish a method superior to the published methods by dissecting the cells and hence

performing a mechanical and enzyme-less isolation of the ICM. Further, the inventors have applied shorter passaging times for yield improval. As apparent from the literature published *after* the publishing of present invention and as discussed, researchers have made use of the method and shown that it is superior to the methods disclosed in the prior art. Finally, the claimed invention provides a useful solution to a technical problem, since the solution has, as discussed above, been adopted by the scientific community working with establishing human embryonic stem cells.

B. At pages 8-10 of the Office Action, claims 4, 9, 10, 60 and 61 stand rejected under 35 U.S.C. § 102(a) as purportedly being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijinders et al., Lanzendorf et al. in further view of U.S. Patnet No. 6,875,607, and further in view of Marshall et al. (Methods in Molecular Biology: Isolation and Maintenance of Primate Embryonic Stem Cells 158:11-18, January 2001).

C. At pages 10-11 of the Office Action, claim 11 stands rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijinders et al., Lanzendorf et al. and in further view of U.S. Patent No. 6,875,607, and further in view of Conner.

D. At pages 11-13 of the Office Action, claims 14-15 stand rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijinders et al., Lanzendorf et al., and further in view of U.S. Patent No. 6,875,607, and in further view of Gardner et al. (1998), when taken with Gardner (1999).

E. At page 13 of the Office Action, claim 35 stands rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Thomson (U.S. Patent No. 6,200,806) when taken with Stratagene Catalog, 1988, p. 39.

Rejections "B"-"E" are respectfully traversed for at least the reasons set forth above in response to rejection "A" above.

In view of the above, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 103.

CONCLUSION


In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this Amendment and Reply or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney so that prosecution of this application may be expedited.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

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By: 
Lisa E. Stahl
Registration No. 56704

Customer No. 21839
703 836 6620